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THE

SPERMATOGENESIS OF AMPHIUMA

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, IN THE FACULTY OF PURE SCIENCE, COLUMBIA UNIVERSITY

BY

J. HOWARD McGREGOR

Reprinted from JOURNAL OF MORPHOLOGY, Supplement to Vol. XV.

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THE SPERMATOGENESIS OF AMPHIUMA.

J. HOWARD McGREGOR.

TABLE OF CONTENTS.

I. INTRODUCTION.

THE investigation upon which the present paper is based was begun in the Columbia University laboratory in the winter of 1896–97, upon material of *Amphiuma* which had been obtained during the preceding September.

Prior to that time several accounts of the spermatogenesis of Amphibia had been published, most of which dealt with the European salamander; and especially noteworthy were two

papers, by vom Rath ('93) and Meves ('96), treating of the later divisions in the male sex-cells of *Salamandra*. The conclusions of these observers were, however, so widely divergent that one of my chief objects was to confirm one or the other of their accounts in the matter of "chromatin reduction"; and, as will be shown beyond, my conclusions regarding this process in the urodele Amphibia coincide almost perfectly with those of Meves. It was my hope also to elucidate the metamorphosis of the spermatids into spermatozoa; for the earlier works of Flemming ('88) and Hermann ('89) left much to be determined, particularly in regard to the origin of the middle-piece and the apical body of the spermatozoön.

The publication of my results has been long delayed, owing to the loss, in August, 1897, of all the material, notes and drawings, when the research was approaching completion; and for many months it was impossible to secure new material. In the mean time appeared the concluding section of Meves's work on *Salamandra* ('97), in which the metamorphosis of spermatid into spermatozoön is so fully and accurately presented that the present paper is, to a considerable extent, merely confirmatory of Meves's results, and my account of *Amphiuma* might, in many places, answer for a description of the spermatogenesis of *Salamandra*. In certain features, however, considerable divergence will be seen to exist between the two forms.

In addition to the material from *Amphiuma*, I have made preparations of the testis of *Necturus maculata*, *Cryptobranchus alleghaniensis*, and of the European *Salamandra maculosa;* but, with the exception of *Cryptobranchus*, none of these was taken in the proper season to show the metamorphosis of the spermatids. For some excellent preparations of *Spelerpes ruber* and *Desmognathus fusca* I am indebted to Dr. Ulric Dahlgren of Princeton University.

To Prof. E. B. Wilson I wish to acknowledge my indebtedness for valuable suggestions and helpful criticism during the progress of the investigation.

II. On the Structure and Structural Changes of the Testis.

In *Amphiuma* the testis is not divided into several large lobes, as in *Salamandra*, *Desmognathus*, and some other genera of urodeles, but consists of a slender spindle-shaped body, as much as 18 cm. in length in a large specimen and, at the season when the sexual products are ripe, usually about 6–8 mm. in diameter near the middle. For the length of 2 or 3 cm. the posterior extremity of the organ is devoid of germinal cells, and is composed of connective tissue and fat. This portion has a dark red color, due, no doubt, to its rich vascular supply, but the greater part of the testis is of a very pale flesh color, passing into white in the portions filled with ripe sperm. The black pigment, common on the surface of the testis in many urodeles (*e.g.*, in *Desmognathus fusca* and sometimes in *Necturus maculata*), is absent in *Amphiuma*.

Along the line of attachment of the testis to the mesorchium is a deep groove or hilum. The surface of the entire organ is finely lobulated, the minute lobules being the outer ends of the seminal tubules, which have the form of long cones radially arranged around the linear center of the testis. The blood vessels pass out, between the tubules, to the surface, which is netted with rosettes of very fine vessels, which are much more conspicuous during the period of actual spermatogenesis. In testes shortly after the breeding season and in those of immature animals the blood supply is much less, as is also the case in those portions of adult testes which are distended with ripe spermatozoa.

In the European salamander, as described by Meves ('96), the testis consists of five distinct regions, and if examined in July or August, the tapering end lobes are found to contain large spermatogonia, while the three intermediate segments, counting from before backwards, are filled, respectively, with small spermatogonia, spermatocytes, and spermatozoa (following the terminology of la Valette St. George). *Amphiuma* does not show this separation into lobes, but the annual spermatogenetic cycle begins near the posterior end of the testis

and progresses very slowly and with great irregularity toward the anterior end, so that at almost any point of the testis a cross-section may contain a number of different stages, contiguous tubules, or even different cysts in the same tubule often showing two or three generations of cells. Usually there is a more or less regular transition of stages between the extreme tubules occurring in a cross-section (*i.e.*, those bordering upon the hilum). At one end of a section may be seen small spermatogonia, while passing toward the other end of the same section we find, successively, primary and secondary spermatocytes and various stages in the metamorphosis of the spermatid.

The *Amphiuma* material at my disposal was killed late in autumn and in the spring (the time of breeding is midsummer), the initial stages of spermatogenesis, — including the "multiplication period" and the "growth period," — which occur shortly after the breeding season, having been completed except in the anterior end of the testes prepared in the fall (October 1st and 20th), which contained a few tubules still filled with small spermatogonia. The large spermatogonia or "Ursamenzellen" are, however, abundant in the deeper part of the testis, at the inner ends of the tubules. These cells are clearly differentiated from all the others of the testis, and it is easy to demonstrate that they arise as proliferations from the epithelial lining of an irregular canal which runs along the center of the testis in the hilum. This canal functions in the breeding season as an outlet for the contents of the tubules, and from it the *vasa efferentia*, lined with ciliated epithelium, pass to the Wolffian duct. The lining of the longitudinal canal in the testis is columnar, but apparently non-ciliated; and there is no doubt that it gives rise to the larger spermatogonia, and therefore represents the germinal epithelium (Pl. IV, Fig. 1).

As long ago as 1876, la Valette St. George stated in the conclusion of his work on spermatogenesis in Amphibia ('76) that "Als Ausgangspunkt der Spermatogenese, sowohl beim Eintritt, als Wiedereintritt der Geschlechtsthätigkeit sehe ich eine mehr oder weniger differenzirte Zellenschicht an, welche die Innenfläche der samenbereitenden Hohlräume des Hodens in der Art eines epithelialen Belages auskleidet und wohl am

passendsten als Keimlager bezeichnet werden mag, da sie für Samenzellen, Samencysten, und Samenfollikel das Bildungsmaterial herzugeben scheint "(*l.c.*, p. 819). The epithelial lining of the canal I have described is doubtless the "Keimlager" of la Valette.

If we compare the so-called "tubules" of the Amphibian testis with the tubules of the Sauropsid or Mammalian organ, it will be seen that the latter are lined throughout by epithelium, which proliferates the germ-cells directly into the lumen, while in the Amphibian the "tubules" contain only the cells which are to produce the current season's crop of spermatozoa, together with some connective-tissue cells. These are all proliferated from the one central tube and are at first solid masses of large spermatogonia, but they multiply greatly, and their progeny enlarging, form the "tubules," which are thus formed anew each year. At the end of the breeding season the old tubules, now containing only connective-tissue cells, and their connective-tissue walls, rapidly degenerate and are absorbed. A testis examined shortly after the breeding time shows a rapid cell proliferation from the center which is to form the new "tubules," while the remains of the old "tubules," filled with the *débris* of degenerating tissue, are seen in the peripheral portion. Thus, as expressed by Meves, "es ist also nicht statthaft hier von Kanälen zu sprechen, wie es die Autoren vielfach thun."

As already stated, the large spermatogonia, after their differentiation from the "Keimlager" of la Valette, lie isolated, each in its proper capsule, in the connective tissue in the deepest part of the testis — the "Regenerationsfeld" of vom Rath. They are the largest cells of the testis, much larger than their parent cells of the "Keimlager." Their proliferation seems to be a gradual process, and when formed they remain quiescent for several months, while the later generations of cells, with which the testis is chiefly filled, are maturing.

When the breeding period is passed and the retrogressive changes are occurring in the empty tubules, the large spermatogonia awake to activity, and mitotic divisions follow each other rapidly, the numerous small spermatogonia derived from one

large one continuing to lie within the capsule of the mother-cell, which thus becomes distended to form a thin-walled cyst of connective tissue, in the wall of which the connective-tissue nuclei still persist. The cysts thus formed are the "spermatocysts" of la Valette, but the contained cells are now universally termed spermatogonia; or, as Meves ('96) has designated them, "kleine Spermatogonien," in distinction from the earlier "grosse Spermatogonien." As the spermatocysts grow they are forced out toward the periphery of the testis by those behind them, so that new radially arranged tubules are formed, separated only by a thin layer of connective tissue. The cysts at first adhere to the wall of the "tubule," leaving a lumen in its center filled only with lymph. This condition is seen for a few weeks after the breeding period, and is also characteristic of immature animals. During the ensuing "growth period" the cells, and consequently their containing cysts, increase in size so that the lumen of the tubule usually becomes completely occluded, and each tubule is then a solid rod of large cells. (Since, as will be shown beyond, the "synapsis" or "pseudo-reduction" occurs during the growth-period, these large cells are "spermatocytes of the first order" or "primary spermatocytes.") The size of the entire testis is also greatly increased at this period.

In *Amphiuma* the growth period occurs late in the summer, the maturation period extending from that time perhaps almost to the breeding season. The spermatocytes near the posterior extremity of the testis mature first, a "wave" of maturation passing very slowly and irregularly toward the anterior tip of the organ. This "wave" occurs in all the urodeles which I have examined, though perhaps not always progressing in the same direction, and by it the season of sexual capability is considerably prolonged. I have noted in *Necturus* in October that the anterior end of the testis was full of ripe spermatozoa, while the other end had long since been emptied, and was already filling with small spermatogonia for the next season's crop of sperm. In this case the new centrifugally growing tubules could be seen in one portion of the testis as they encroached upon the old tubules still filled with spermatozoa. *Crypto-*

branchus alleghaniensis, if examined in July, also shows a wave of maturation passing from the posterior to the anterior end, but in this species the maturation divisions and the metamorphosis of the spermatid are accomplished with great rapidity, just prior to the breeding season, so that the spermatozoa are matured in the latter part of August, barely in time to fulfill their function.

The difference in the season and duration of the maturation period in different urodeles—even between some which in general mode of life are not greatly dissimilar—is worthy of some attention. In *Diemyctylus viridescens*, for example, oviposition occurs in the spring, but sexual union may occur at almost any time late in the fall or in the spring, and the spermatozoa are sometimes retained alive for weeks or months in the spermatheca or in the cloaca of the female.[1] The male has the *vas deferens* full of ripe sperm for several months, and the same is true of certain portions of the testis, at least from October to April inclusive. In *Diemyctylus*, accordingly, spermatogenesis occurs during the summer, and there is a constant supply of mature spermatozoa throughout the winter. In *Necturus* also, according to Kingsbury, sexual union occurs in the autumn, the female retaining the sperm until the time of oviposition, which is in May. I have not ascertained the season at which the maturation phenomena occur in *Necturus*, but it is probably early summer. *Cryptobranchus alleghaniensis* deposits its eggs in August and September, but here the sexual union must occur very near or at the time of egg-laying, for the female is devoid of spermathecae, and the spermatozoa do not ripen until late in August. Specimens examined early in August had no spermatozoa in the *vas deferens*, though the testes appeared full almost to bursting. By the end of August all the adult males examined had the *vas* full, but others killed in October show the ripe spermatozoa to have disappeared entirely, the testis, now shrunken to perhaps one-sixth of its former bulk, being filled with small spermatogonia.

We see from this that *Cryptobranchus* is somewhat of an exception to the general rule among urodeles, in that its male

[1] *Cf.* Jordan ('93).

sexual elements are formed barely in time to subserve their function, while in most species they are formed much earlier and are retained in a mature state for weeks or months, either in the male or in a special portion of the female genital passages. The mere fact that a difference exists between different forms, in regard to season and duration of the maturation period, is of little intrinsic interest, but if we connect with this the difference in size of the middle-piece of the spermatozoön in the various forms, we may find a possible connection between the two. When we note that most of the urodeles in which the spermatozoa are formed long before they are to be used have the middle-piece of large size, and, further, that *Cryptobranchus*, which matures its sperm barely in time for use, has an extremely small middle-piece, the contradistinction suggests that size of middle-piece and length of life of the spermatozoön may be causally connected. It is, perhaps, worth suggesting that the greater part of the middle-piece may function as a sort of storehouse of energy, enabling the spermatozoön to retain for a time its power of motion, the duration of this power depending upon the size of the middle-piece.[1]

According to my own observations on *Amphiuma* (given in detail beyond), the greater part of the middle-piece is here not of centrosomatic origin, but probably comes from the sphere (*Idiozome* of Meves). That such observations are in accord with the behavior of the middle-piece in fertilization is indicated by the conclusions of Fick ('93), based on his studies of fertilization in Axolotl. He says: "Anderseits ist es aber ebenfalls höchst unwahrscheinlich, dass das ganze Verbindungsstück (middle-piece) ein etwa in die Länge gezogenes Centrosoma darstellen sollte, da es viel zu gross dafür ist. Endlich könnte man auch noch an Archoplasma denken."

Opposed to my suggestion is the fact that, so far as we know, the middle-piece does not diminish in size or alter in its staining reactions after the adult form is attained. Nevertheless, when two genera, like *Necturus* and *Cryptobranchus*, so nearly similar in their mode of life and in the size of their eggs,

[1] *Cf.* the views recently expressed by Zimmermann ('98), von Lenhossek ('98), and Henneguy ('98).

exhibit such an enormous difference in the size of the middle-piece, one is tempted to correlate it with the great difference in the length of life of the spermatozoa.

III. The Spermatogonia.

Since the names "large" and "small" spermatogonia, as employed at present, indicate nothing in regard to their genetic relationships, I shall use instead the terms "primary" and "secondary" spermatogonia, the former referring to the large individual parent cell, the latter to the smaller daughter spermatogonia occurring in the cysts. It is perhaps needless to explain that all secondary spermatogonia do not belong to the same generation; some may be the immediate progeny of the primary cell, while others are removed from the latter by several generations. Meves ('96), recognizing this fact, speaks of the "Spermatogonien mittlerer Grösse kurz nach Beginn der Cystenbildung," and of the "kleinen Spermatogonien" which fill the same cysts later. In the following discussion I shall treat the primary and secondary spermatogonia in their natural sequence.

A. *Primary Spermatogonia.*

a. *Polymorphism and Amitosis.*—The primary spermatogonia surpass in size all other cells in the testis, frequently attaining a diameter of 50 μ. In contour they are nearly spherical, and in section do not exhibit the polygonal outline, due to pressure of adjoining cells, which is seen in the later generations of sex-cells. The character which first arrests attention in these cells is the form of the nucleus, which is sometimes spherical, but commonly very irregular, being frequently reniform, while in some cases it is in two separate parts. Often, again, the nucleus shows a number of lobes almost constricted off from its main mass. Such nuclei are the "polymorphic forms" described by a number of workers on the amphibian testis. La Valette St. George ('76) noted these nuclei in spermatogonia of *Rana*, in which he described them as dividing into a greater or less number of pieces, segments being constricted off from the

surface, and he aptly compares their form to that of raisins or mulberries. Though I find nuclei in *Amphiuma* in various stages of binary division by simple constriction, they do not undergo complete multiple fission; however deep the constrictions, the small lobules are never entirely separated.

Though both Meves ('91 and '94) and vom Rath ('91 and '93) find amitosis occurring in these cells in *Salamandra*, their observations differ widely as to the "mode" of direct division, particularly regarding the rôle which the "archoplasm" or "sphere" plays in the process.[1] The difference of opinion of these two observers is, however, of little importance when compared with their divergence in the interpretation of the process. On this question Meves states positively, and apparently upon good evidence, that amitosis is a normal process in the spermatogenetic cycle, and that the polymorphic nuclei later regain their smooth contours and divide by ordinary mitosis, and that the daughter-cells produced by amitotic fission likewise come to divide mitotically, giving rise eventually to functional sperm-cells.

With all of these points vom Rath's conclusions are at variance. He asserts that the polymorphic forms do not belong in the developmental cycle, that they are only cells in process of degeneration, and, at most, can but function as nutritive material for the normally developing sex-cells.

Though cells showing amitotic division are fairly abundant in my material, none of them shows the slightest evidence of the ring-shaped centrosphere of Meves, nor of the type of sphere figured by vom Rath. As to the more important question, namely, whether amitosis does or does not signify degeneration, my observations, on the whole, support Meves's interpretation. If the polymorphic nuclei are to degenerate, why should they be so numerous in the regenerating part of the testis? for at certain seasons nearly all the primary spermatogonia have nuclei of this type. And further, if these and the

[1] Meves describes and figures the nucleus as being "cut in two" by the apparent constricting power of a ring-shaped centrosphere, while vom Rath, working, in part, upon similar material, fails to find any indications of such a ring-shaped structure.

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amitotically produced cells are all destined to disintegration, why is not the *débris* of such dissolving elements to be seen? As a matter of fact, they never appear to "degenerate" below the stage of polymorphic nuclei, though all gradations between these and the cells with smooth "normal" nuclei are demonstrable.

Since the polymorphic form in *Salamandra* occurs in greatest abundance in the winter months, the view first expressed by Bellonci ('86), that polymorphism is a consequence of lack of nutriment, is still held by most observers. Hermann ('89) thus calls them "Hungerzellen," but goes farther than Bellonci, expressing the belief that the polymorphic form is assumed during increased metabolism, the increased nuclear surface affording greater opportunity for chemical interaction between nucleus and cytoplasm. In *Amphiuma* the polymorphic nuclei occur in early autumn and also late in the spring, and at all times a few cells of the smooth-nucleated type are scattered among them. Evidence regarding the mechanics of amitosis is totally lacking in my material; a simple cleft seems to cut the nucleus into two nearly equal parts. The only spermatogonia dividing by amitosis are those with reniform or dumb-bell-shaped nuclei; the mulberry-shaped nuclei represent only a "resting stage." I have been quite unable, in amitotically dividing nuclei, to discover the presence of centrospheres, either of the ring form described by Meves or the spherical form of vom Rath.

b. *Finer Structure.* — The primary spermatogonia, by reason of their great size and the weak stain of the nucleus, stand out with great distinctness from the surrounding elements. By far the greater number show the polymorphic type of nucleus, but the internal structure of the nucleus is much the same, whatever the character of its surface. In all cases the nuclear membrane is distinct and takes the plasma stains deeply. Internally the nucleus shows a somewhat ill-defined linin reticulum, suspended in which are a varying number of small masses of chromatin. In general, the larger or the more deeply cleft the nucleus, the fewer and smaller are the chromatin-masses and the more feebly do they stain. In some of the

larger polymorphic nuclei, scarcely any portion of the contents shows affinity for the specific chromatin dyes. In preparations stained with Heidenhain's iron-haematoxylin it is difficult to say with certainty what portions of the nucleus represent chromatin, since all nuclear structures are stained a diffuse gray. The case is different in material double-stained with tar colors, and the best results in the study of the resting nuclei were obtained by Auerbach's methyl-green-acid-fuchsine method after fixation in acetic alcohol. (This stain also gives brilliant results after fixation with Hermann's fluid, but the coloring is then rather transitory.) In such preparations the chromatin, dyed green, is clearly differentiated from the linin, which takes the red acid-fuchsine. The contrast in such preparations between the spermatogonium nuclei and the connective-tissue nuclei, which lie thickly interspersed among them, is most striking; the amount of chromatin in the spermatogonia being so sparse that, except under high powers, the entire nucleus appears to be made up of the red-stained achromatic structures. In the oval connective-tissue nuclei, on the contrary, the chromatin is so abundant that it seems to compose the entire nucleus, the linin network being discernible only with high powers. Meves ('94) mentions in spermatogonia the "mehrfache Chromatinbrocken, die in keinem Zusammenhang mit einander stehen," but since all his material was fixed in strong osmic mixtures, he deems it probable that only the more dense masses of chromatin can be demonstrated, owing to the destructive "osmication" of the tissue. My material fixed in acetic alcohol shows clearly the linin fibers running from one to another of the chromatin-masses, and apparently also from them to the nuclear membrane, and the same is shown, though less clearly, in testes fixed in Hermann's fluid. In Meves's later work ('96) he, too, finds the "Lininfäden welche die Chromatinbrocken untereinander in Zusammenhang setzen" in material killed in Flemming's and Hermann's fluids. Meves also describes one or more large nucleoli, usually surrounded by a clear space. In only a few primary spermatogonia have I found such appearances, but they are so rare that I incline to consider them artefacts. One constantly sees, however, small bodies in the

nucleus which take the plasma stain, but they appear to be mere condensations of the linin structure, and their number is inconstant.

All of the foregoing description of the resting nucleus applies equally to spermatogonia with polymorphic and spherical nuclei, the only difference between the two forms being in the size, number, and staining power of the chromatin-masses. In the spherical nuclei the latter are larger, more numerous, and stain more brilliantly.

Though there is so little difference in the intimate structure of the nuclei, the cytoplasm of the two general types of primary spermatogonia shows some difference of constitution, the cells with polymorphic nuclei (this term here including the nuclei dividing by amitosis, as well as those of the mulberry form) showing no sphere (idiozome), while those with "normal" nuclei possess a somewhat ill-defined sphere. The cytoplasm in these cells is less voluminous than in the polymorphic type and shows a denser portion massed on one side of the nucleus, imbedded in which is the small, rather homogeneous sphere. I have not been able to demonstrate centrosomes within it.

B. *Secondary Spermatogonia.*

As explained above, the term "secondary spermatogonia" includes those grouped in cysts, each of the latter containing the descendants of one primary spermatogonium. The account already given of the primary spermatogonia would answer in many particulars for these; it will therefore suffice to give the points in which they differ. In the first place, the secondary spermatogonia are much smaller, but of different sizes, according to the number of generations by which they are removed from the primary. In form they are usually angular, from compression, and, especially in young cysts, they may have, roughly speaking, a conical or pyramidal shape, the base of the cone or pyramid facing outward toward the cyst wall. In such cases the nucleus, always approximately spherical, — for the nuclei of secondary spermatogonia are never polymorphic, — lies near the outer end or base, while the bulk of the cytoplasm

is at the apex. In older cysts, where the cells are several layers deep, the form is less regular. The structure of the nucleus appears to be exactly similar to that of "normal" primary nuclei, unless, perhaps, the chromatin lumps are here larger and closer together. The cytoplasm is small in amount and a sphere can be seen lying in the densest part, but like that of the primary spermatogonium, it is small and ill-defined, and shows no inner and outer zone. Within it are two excessively small centrosomes. (The centrosomes are smaller in every stage in *Amphiuma* than in *Salamandra.*)

When the cells of a cyst happen to be so placed that the spheres of two or more contiguous spermatogonia lie within the plane of section, it is sometimes possible to discern "bridges" which unite the spheres of neighboring cells (Pl. IV, Fig. 3). At the points of transit from one cell to another there are distinct mid-bodies (Zwischenkörper) which have the form of two rings in close apposition, each ring of the pair lying in the plane of the cell-membrane of its cell. These ring-shaped mid-bodies are plainly formed by splitting of a single mid-body, and occasionally the two rings of a pair may be some distance apart. The "bridges" are to be regarded as remnants of central spindles, and when a cell contains two or more bridges, each one represents a past mitotic division. The fibers composing the bridge diverge, sheaf-like, at the ends and do not end in the spheres, as do the bridges figured by Rawitz ('95) and others.

a. *Mitosis of the Secondary Spermatogonia.* — In *Amphiuma* primary spermatogonia in the resting condition, and dividing by amitosis, were found in material prepared in October and also in May; the secondary spermatogonia were found only in the material prepared in October, and there only in the anterior tip of the testis. Of the primary spermatogonia none were found dividing by mitosis, and of the secondary only a very few. The period in which to find spermatogonia actively dividing (the "multiplication period") is shortly after the breeding time, or in midsummer, and no material was available at that season.

A few secondary spermatogonia were found in the early pro-

phase, showing the spireme, which differs chiefly from the corresponding stage of the spermatocyte in being more contorted. Another and more important difference is that here the spireme threads remain single even after the nuclear membrane has disappeared, while in the prophase of the first spermatocyte division a longitudinal splitting of the chromosomes occurs while the membrane is still intact. Of the few spireme stages found in secondary spermatogonia, none showed sphere or centrosomes, these organs lying doubtless without the plane of the section. One group in a later prophase showed the two diverging centrosomes, and between them the central spindle. The appearance of these cells is represented in Pl. IV, Fig. 4, and in view of the fact that similar stages of *Salamandra* have been fully discussed and figured by Drüner ('95) and Meves, it is unnecessary to describe them here. It may be stated, however, in regard to the number of chromosomes, that it is plainly greater than in the first spermatocyte division, which is known to be post-synaptic, *i.e.*, after the pseudo-reduction. As will be fully explained beyond, in connection with "reduction," the post-synaptic number of chromosomes in *Amphiuma* is *twelve*, thereby agreeing with *Salamandra*. From supplementary observations on *Spelerpes ruber* and other urodeles, I am certain that the spermatogonium divisions show the normal somatic number, or twice as many as the post-synaptic stage. My observations are thus in harmony with those of Meves, as opposed to vom Rath ('93), who claims to have found the reduced number even in the larvae.

b. *The Growth-period and Synapsis.* — After the period of multiplication has come to an end, the cysts are closely packed with small secondary spermatogonia, in a condition of rest, and these now, without farther division, become transformed into much larger and very different cells — the primary spermatocytes. The period during which this takes place is the "growth period."

The main structural changes incident to the growth-period occur in the nucleus. The chromatin, which up to this time was disposed in small isolated lumps, now begins to form amorphous granular masses and strings, at the same time increasing in

amount. In the midst of this shapeless mass of chromatic and achromatic material, one or two large vacuoles containing nucleoli are frequently visible. This condition gradually merges into that shown in Pl. IV, Fig. 5. The granules become united into globules of uniform size, and the latter become arranged in a long coiled thread, or more probably several threads. Under the higher powers of the microscope these threads exhibit irregular lateral projections which give them a "mossy" appearance. This is due partially to the linin meshwork and partly also to the fact that fine "Brückenfäserchen" (to borrow Hermann's term) connect the adjoining loops of the wreath of chromatin. These bridge filaments are composed of minute granules of chromatin suspended in threads of linin substance. Many of the chromatin loops are in close contact with, and apparently adherent to, the nuclear membrane at this stage, though by no means all the chromatin is thus peripherally distributed. The nucleus now contains one, or sometimes two, large nucleoli which stain brilliantly with the plasma dyes, and are always surrounded by a clear vacuole.

While the above-described nuclear changes have been taking place, the cell has grown to perhaps twice its former volume, and the increase in the cytoplasm has kept pace with the growth of the nucleus or even exceeded it. The cytoplasm, which now shows much better general preservation than in earlier stages, consists of a close, even meshwork, extending throughout the cell. The nucleus occupies an excentric position on the side nearest the cyst wall, so that the great bulk of the cytoplasm lies on the side toward the center of the cyst. In the midst of this mass of cytoplasm lies a well-defined sphere (idiozome), in which three distinct zones — an inner, middle, and outer layer — are discernible. The central part of the sphere is dense, and imbedded within it are two minute centrosomes; the intermediate zone surrounding this is much clearer, but the third or outer zone is a thin layer of the denser sphere-substance similar to the central portion, and its surface is usually beset with slight projections. A few slender bars of the dense sphere-substance traverse the intermediate zone radially, thus connecting the inner and outer zones. A greater or less num-

ber of dark granules are observable in the outer layer, as well as in the deeper portion of the sphere, but the outer zone has no appearance of being a zone of microsomes.[1]

The intercellular "bridges" and "Zwischenkörper," described as occurring in secondary spermatogonia, still exist and are more distinct than before the growth-period. Fig. 6 represents three cells united by bridges. The double-ring mid-bodies are larger than in the spermatogonium stage.

At some time between the beginning of the growth-period and the prophase of the first maturation division occurs the reduction in the number of chromosomes to one-half the somatic number. To this phenomenon the name pseudo-reduction (Scheinreduction) was given by Rückert. It was first noted in Amphibia by Flemming ('87), and appears to be of universal occurrence in sex-cells of both animals and plants.

Moore ('95), in his studies of spermatogenesis of elasmobranchs, lays special stress on the fact that the actual halving of the number of chromatin-masses occurs while the nuclei are still at rest (in the growth-period), and for the period at which this occurs he proposes the term "Synapsis." The difference in the number of chromosomes is the *essential* difference between a spermatogonium and a spermatocyte, but it scarcely need be explained here that synapsis is not "reduction" in the Weismannian sense. It is difficult to discover in *Amphiuma* exactly when this pseudo-reduction occurs, but from examination of a large number of cells, I incline strongly to the belief that the spireme arises from the preceding stage, with the chromosomes already formed in the reduced number. Synapsis then may be said to occur at about the end of the growth-period, at the

[1] The appearance here described is that of the spheres in the parts least exposed to the destructive action of fixatives, and it will be seen to closely resemble the spheres figured by Meves. (*Cf.* Meves, '96, Fig. 41, with Figs. 6 and 7 of the present article.) A curious effect of osmication was noted in some peripherally lying cells of this stage in a preparation of *Spelerpes ruber*, which had been fixed in Flemming's chromo-aceto-osmic mixture. The effect of osmication was to give the sphere the appearance of a clear vesicle, in the center of which was a very pale homogeneous body enclosing the two centrosomes. The cytoplasm immediately surrounding the sphere was filled with granules, which, like the centrosomes, stained intensely with iron-haematoxylin. A cell from this preparation is represented in Pl. IV, Fig. 6.

time when the spireme is formed. A division of the spireme threads by longitudinal splitting occurs later, thereby differing from the mode of formation seen in *Ascaris* by Brauer ('93), in which a continuous spireme first splits longitudinally, and later breaks into chromosomes.

The changes above described as occurring in the growth-period in *Amphiuma* parallel pretty closely those which Flemming ('87) and Meves ('96) have described in *Salamandra*, though there are some minor points of difference; for example, the small rods, staining intensely with iron-haematoxylin, which the latter author describes in the substance of the sphere, are entirely lacking. (Somewhat similar "Archoplasmaschleifen" are figured by Hermann in Helix, '91.)[1]

The cells in the above-described spireme stage are primary spermatocytes, and in this condition they rest for a considerable period, probably several months, as is evidenced by the great abundance of such cells from October to May inclusive. This stage is represented in Pl. IV, Fig. 5.

IV. Maturation (Spermatocytes).

The condition of rest in which the primary spermatocytes were last described gives place finally to another division, the first of the two maturation divisions. By these two mitoses the chromatin is distributed to the spermatids, and in those forms in which "reduction" or qualitative division of chromatin occurs, that process is accomplished by these two mitotic divisions. In *Amphiuma* no true "reduction" occurs, and the maturation phenomena are, in general, very similar to those described by Meves ('96) in *Salamandra*; *i.e.*, there occur two longitudinal divisions of the chromosomes (the synapsis or

[1] Rawitz ('95) describes the spheres in *Salamandra* as homogeneous throughout, which condition is undoubtedly due to the methods employed, since the other investigators find the zones well marked. Rawitz also states that but one centrosome is present, which is obviously an error, since both Meves and myself find them divided before the growth-period begins. The condition of the chromatic structure, as represented in Rawitz's figures, indicates a rather early phase of the growth-period, which may explain the homogeneity of his spheres. The jagged outline of the sphere in his Figs. 3 and 4 closely resembles those I have described.

pseudo-reduction above mentioned has, of course, reduced the number of chromosomes to twelve, or one-half the normal number) but no transverse divisions. The chromatin of the four spermatids derived from each primary spermatocyte by these two divisions is therefore exactly equivalent qualitatively.

A. *The First (Heterotypical) Maturation Division.*

The first maturation mitosis does not occur simultaneously in all the primary spermatocytes, but progresses slowly, involving only small groups of cells here and there, and even a single cell in a cyst may divide, while the others remain at rest in the spireme stage. The actual mitosis probably requires a brief time, but since so few cells are involved at the same time, several months are required for all to mature.

The rough spireme of the resting nucleus becomes smoother, but before the threads have quite lost their moniliform appearance a longitudinal splitting occurs, as described by Flemming ('87). By the time the chromosomes are distinctly split they are quite smooth. The separation of sister-chromosomes is at first complete, but a little later they are seen to be united at both ends so as to form elongate rings, and these, becoming shorter and thicker, are thrown into a figure-of-8 form, or even twisted into a loose rope.[1] These loops are the "heterotype" chromosomes of Flemming ('87). Their general position in the nucleus is peripheral, and they show a tendency to lie in planes which cut the sphere (Pl. IV, Fig. 7).

While the longitudinal splitting of the chromosomes is occurring, changes are also taking place outside the nucleus; namely, the appearance of a very fine radiation surrounding the sphere and extending almost or quite to the cell-membrane. Slightly later the two centrosomes within the sphere gradually move apart, and it may then be seen that the radiation is double, each centrosome being surrounded by an aster. The

[1] Flemming describes some of these in *Salamandra* as having the sister-threads united primarily at only one end, the other ends uniting later to form a closed ring, but this I have not observed in *Amphiuma*. The cut stumps of chromosomes, which are always in evidence in thin sections, might readily be mistaken for ununited ends.

zones of the sphere are in some cases clearly distinguishable after the centrosomes are widely separated, and the substance of the sphere evidently takes no part in the formation of the astral rays. Fig. 8, which represents this stage in *Spelerpes ruber*, shows a well-defined homogeneous sphere and widely separated centrosomes with distinct radiations. Fig. 7, from a spermatocyte of *Amphiuma*, shows a sphere with distinct, inner, middle, and outer zones, but the centrosomes have come to lie at its periphery; and frequently at this stage they lie entirely outside the sphere, between it and the nucleus. In such cases there is usually a break at one side of the sphere, presumably in the region where the centrosomes and their connecting "centrodesmus" made their exit.

Such spheres as these, which are not a part of the aster but are merely traversed by it, cannot be regarded as spheres of microsomes, as they were described by Drüner ('95) in *Salamandra* spermatocytes (and by Heidenhain, '94, and Van Beneden, '83, in Amphibian leucocytes and Ascaris eggs respectively); nor are they part of a persistent "Archoplasm" in the sense in which Boveri originally used the term, since they have, in this case, no part in the formation of the achromatic structures of the new mitotic figure.[1] The chief difference between the sphere in this stage in *Salamandra* (as described by Meves) and *Amphiuma* seems to be its greater persistence in the latter form; for while in *Salamandra* it breaks into irregular masses which later become spread out over the surface of the nucleus before the centrosomes have separated, it may persist in *Amphiuma* and even continue to retain the three zones after the spindle has attained a considerable size (Pl. IV, Fig. 9). Later, however, the sphere disintegrates and its fragments are visible lying outside the spindle until the end of the anaphase.

The formation of the central spindle occurs essentially as described in *Salamandra* by Hermann, Drüner, and Meves. The very young spindle lies close against the nuclear membrane, but is usually imbedded in a slight concavity, so that in

[1] On this point my observations entirely agree with those of Drüner ('95) and Meves ('96).

many cases the actual spindle cannot be seen, and only the radiations indicate the positions of the centrosomes. The latter, when visible, are much smaller than in *Salamandra.* The concavity in the nuclear wall, in which the early spindle lies, is probably caused, as Meves suggests ('96, p. 46), by a pushing of the spindle against it, in consequence of the lengthening of the pole-fibers.

The ring-shaped chromosomes become considerably shorter and thicker during the growth of the central spindle. Commonly they become most thickened at the ends, where the union of sister-threads occurs, and in some cases the latter become fused for some distance from the ends. Toward the middle they become widely separated, appearing sometimes as rings while the spindle is still quite small, but usually they are considerably twisted. Until the disappearance of the nuclear membrane, which occurs later in *Amphiuma* than in the European *Salamandra,* the chromosomes retain their peripheral position, being most thickly grouped on the side toward the spindle. At this time the linin reticulum is quite conspicuous, and is densely massed in the center of the nucleus, becoming more sparse toward the periphery. The disappearance of the nuclear membrane, as in the salamander, is accompanied by an aggregation of the chromosomes at the side of the nucleus farthest from the spindle. Hermann ('91, p. 573) accounts for this by supposing "dass dort wo das Archoplasma dem Kerne anliegt, in den Prophasen der Karyokinese zuerst die Selbständigkeit des Kernes gegenüber dem Zellleib gelockert wird und dass dann von dieser Stelle gewisse Flüssigkeitströmungen in das Innere des Kernes eindringen." A more credible explanation is that of Drüner ('95), who asserts that upon the breaking of the nuclear membrane the chromosomes are pushed away from the spindle by the same rays which later become the contractile mantle-fibers.

From this time forwards the development of the achromatic figure is so similar to that described by Hermann that it need be given here only in broad outline. As the central spindle increases in length the polar radiations become, for a time, more distinct and may be seen extending quite to the cell-

membrane, and before it has attained more than one-third of its full length the mantle-fibers are readily distinguishable.

Regarding the origin of the mantle-fibers two widely divergent accounts have been given; one of these, enunciated by Hermann ('91) and supported by Drüner, is that they are differentiated from astral rays and grow into the nucleus after the nuclear membrane has become broken. The other view, held by Flemming ('91) and Meves ('96), is that they arise from the linin reticulum of the nucleus. The point is not an easy one to determine, but I believe that in *Amphiuma* the mantle-fibers arise, as Hermann maintains, from the aster.

The stage illustrated in Fig. 10 shows the heterotype chromosomes drawn into position on the spindle, and it is now possible to see clearly that the mantle-fibers are attached, not to the original ends, but to the middle of each chromosome, and at these points of attachment the sister-threads become drawn away from each other in the form of V's; the ends of the V's by which they remain united are the ends of the original threads of the double-spireme stage, while the apices of the V's represent the middle points of those threads. The great difference in the size and form of the chromosomes is noteworthy; it is often much more marked than in the cell shown in Fig. 10. In cells which are cut in the equatorial plane it is now possible to count the chromosomes, which, as in other urodeles, are twelve in number. I have never been able to count the chromosomes in spermatogonia, but since their number in those cells is plainly much greater than twelve, it is safe to assume that in this form, as in *Salamandra*, the pre-synaptic number of chromosomes is twenty-four.

Pl. IV, Fig. 11, illustrates the anaphase of the heterotypical mitosis. There is some irregularity in the time of separation of the daughter-chromosomes, some having approached the poles quite closely, while others are still united.

The second longitudinal split (equation division) occurs about the time of separation of the V's. This division is first discernible in the knobbed ends, which lie near the equator of the spindle, and it passes thence along both V's toward their apices (*i.e.*, from the original ends of the chromo-

some toward the middle), but the longitudinal split is not always completed when the V's break apart at their ends. During the late anaphase each pair of sister-V's formed by the last longitudinal split remain in close contact.

During the anaphase the spindle increases considerably in length (*cf.* Figs. 10 and 11), and I incline to agree with Drüner ('95) that this lengthening is caused by growth of the spindle fibers, which thus *push* the centrosomes wider apart. The astral rays which connected the centrosomes of the young spindle with the cell-membrane have almost disappeared when the anaphase begins, only a little of their central ends being visible.

In the telophase the dispireme condition is attained, as described by Meves.

As division of the cell-body proceeds, the central spindle persists as a delicate bridge, its fibers showing the thickenings which later fuse to form a ring-shaped mid-body. A new radiation, the astral shield (*Strahlenschirm*), surrounding the centrosome now replaces the aster, the latter having disappeared during the anaphase. Meves figures the centrosome as divided during these changes ('96, Figs. 61–70), but in *Amphiuma*, owing to its minuteness, this point cannot be determined. The centrosome, with the shield, migrates through an arc of approximately 90 degrees, so that the axis of the shield and that of the "bridge" are almost at right angles. The astral shield appears as a very broad pencil of rays, proceeding from a point close to the cell-membrane; the other ends of the rays are attached to the cell-wall.

The daughter-cells (secondary spermatocytes) finally come to rest with the nuclei in a rough spireme (Pl. IV, Fig. 12).

The chief difference between such a cell and the corresponding stage in *Salamandra* is that there is here a compact sphere instead of "eine verschiedene grosse Zahl homogen-aussehender Ballen und Brocken von Sphärensubstanz, neben oder zwischen welchen die Centralkörper liegen" (Meves). I have not been able to demonstrate centrosomes inside the sphere, but about its periphery are always several deeply staining granules, one or two of which are doubtless centrosomes. Mid-bodies and

very delicate spindle bridges are visible, but the latter do not appear to come into relation with the spheres. In general appearance the secondary spermatocytes resemble the primary, but are much smaller. The main structural difference is in the spheres, those of the secondary spermatocytes never showing concentric zones, and never containing the centrosomes imbedded in them. The spheres are of similar (astral) origin in both generations, and in the secondary spermatocytes the astral shield of the preceding telophase appears to be metamorphosed directly into the sphere.

These secondary spermatocytes, with a well-marked spireme in the nucleus, and with a compact sphere, represent a more complete resting stage than has been described in other Amphibians, but as this stage occurs but sparsely, it is perhaps of short duration.

B. *The Second (Homœotypical) Maturation Division.*

The stages of the final division are sufficiently elucidated in the figures (Figs. 13–17), which require little explanation. The behavior of the achromatic parts is, in general, the same as in the preceding mitosis. The chromatin emerges from the spireme in the form of twelve V's longitudinally split, which are probably identical with those of the anaphase of the preceding division, though this cannot be stated with absolute certainty, for it is impossible to discover exactly how the new double V's arise from the spireme. To all appearances, the spireme gives rise to twelve rather thick chromosomes, which become bent, and then split longitudinally, the halves remaining in close contact to form double V's. Thus, in all essential features the maturation of *Amphiuma* corresponds with that of *Salamandra* as described by Meves.[1]

Several writers have attempted to show how two longitudinal

[1] The qualitative reduction which vom Rath ('93) describes in *Salamandra* is, I believe, quite without support. The tetrads (Vierergruppen) and dyads (Zweiergruppen) which he figures are either artefacts or the stumps of double and single V's which have been cut in making the sections. The stumps of such cut V's do occasionally resemble tetrads. The errors in vom Rath's account are fully indicated in Meves's paper ('96).

divisions might result in a true reduction in the Weismannian sense; and even Meves states (*l.c.*, p. 64) that at first he sought to show that the *ends* of the sister-chromosomes, and *not* their *mid-regions*, were drawn toward the poles in the heterotypical mitosis, a process which would result in a true reduction, but he was forced to admit that this was not the case. Kingsbury ('99), in a paper on *Desmognathus fusca*, finds the two equation divisions, as described by Meves, but points out a possibility of true reduction in the final mitosis. He states that "if the second division in *Desmognathus* is to be looked upon as a 'reducing division,' it may be considered in two ways. Either the original union of the chromosomes after two longitudinal splittings of the united chromosomes is dissolved, and a new union between the daughter-chromosomes established; or, from the standpoint of the more typical mode of reduction by tetrad formation with longitudinal and transverse divisions, there would occur in *Desmognathus* a reduction in number to one-half, a longitudinal (equation) division, followed by an attempt at a second longitudinal division, which, however, is not completed, and which is prevented from being completed by the second division, which is transverse." This second hypothesis, however, does not accord with the known facts, as Kingsbury observes them in *Desmognathus*, and it certainly does not hold in *Amphiuma*. That two longitudinal divisions occur in *Amphiuma* is quite certain, and the only possibility of a true reduction *after* these divisions is by conceiving the chromosomes to be "bivalent" in Rückert's sense, and to assume (Kingsbury's first hypothesis) that the halves of these bivalent chromosomes form new connections before the final mitosis. This interpretation was suggested to Kingsbury by the presence in the prophase of the second division of $\times$- or $+$-shaped chromosomes. For an $\times$-shaped chromosome the formula would be ${}^{a}_{a}\!\times\!{}^{b}_{b}$; if, now, it be divided horizontally, the sister-V's will each have the formula *ab*; a vertical division, however, would give two V's with the formulæ *aa* and *bb*, which would represent a true reduction.

This hypothesis is somewhat difficult to apply in the case of

Amphiuma, where no X-shaped chromosomes are observable; but a rearrangement of the chromatin of the sister-V's in the interval between the two mitoses must be admitted as a possibility. It seems more probable, however, that the double V's of both mitoses are identical, both representing the same equation division.[1]

The telophase of the final division is very similar to that of the preceding mitosis. The centrosome migrates in the same manner and becomes surrounded by an astral shield, but in this case the centrosome comes to lie in close contact with the cell-wall. The dispireme condition is illustrated in Pl. V, Fig. 17. This nucleus now increases in size, and the chromosomes become distributed peripherally (Fig. 18), but they soon become merged into a rough spireme (Fig. 19).

The cells produced by this second and final maturation division are spermatids. Since the distribution of chromatin is accomplished by two longitudinal (equation) divisions, there is no "reduction" in the Weismannian sense, and of the four spermatids derived from one primary spermatocyte, all are exactly equivalent as regards their chromatin.

V. Metamorphosis of the Spermatid.

A. *Early Changes: Differentiation of Axial Filament, End-knob, and Ring.*

The condition last described, with the chromatin disposed in a rough spireme (Pl. V, Fig. 19), may be considered the starting-point of the transformation of spermatid into spermatozoön.

[1] From studies on the egg of the urodele *Triton*, Carnoy and Lebrun ('99) arrive at conclusions essentially similar to those of Meves and myself regarding the "reduction" question, though their account of the details is widely different. After the chromosomes have become arranged on the spindle, there occur two longitudinal splittings, the first equatorial, the second axial, though neither results in complete separation. The next step is an elongation of this "tetrad" in the equatorial plane so that the original ends come to lie at the middle of a transverse rod of chromatin, while the original equatorial split, by which the chromosome elongated axially, becomes obliterated. At the middle point (the original ends) the half chromosomes break apart, thus completing the *axial* cleft. The halves next bend into a V shape and become superimposed to form a double V, but later they separate, one going to each daughter-cell (egg and first polar body). In

The spireme condition gives the cell the appearance of being about to divide by mitosis. The centrosome, which lies close against the cell-membrane, still possesses its conical "astral shield," and in the cytoplasm near it can usually be seen remnants of the sphere-substance of the mother-cell. These are the same masses which, during the anaphase, were visible lying outside the spindle.

Judging from the paucity of cells which exhibit it, this stage of the spermatid is of brief duration. The first change observable is the gradual disappearance of the astral shield, accompanied by the appearance, between centrosome and nucleus, of a rounded mass of sphere-substance. There can be little doubt that this sphere is formed by direct metamorphosis of the astral shield, though the masses of old sphere-substance which persisted throughout the preceding mitosis also contribute to it. By the time the sphere is well formed, the centrosome is seen to be double (it probably divides much earlier), the two parts being connected so as to form a dumb-bell-shaped body. One of these centrosomes is in contact with the cell-wall, the other, more deeply placed, is connected with the sphere (Pl. V, Fig. 20). This condition of the centrosomes is quite similar to that figured by Moore ('95) in elasmobranchs, Meves ('97) in *Salamandra*, and by some other investigators. In some spermatids the cell-wall shows a slight pit at the point where the outer centrosome is attached.

The rough chromatin spireme now disintegrates into a number of large and small masses placed in the utmost irregularity (Fig. 20). These rapidly break up into finer granules, so that the nucleus soon assumes the appearance of a great number of chromatin grains of various sizes, but all small, suspended in a linin reticulum. Within this, somewhat excentrically placed as a rule, are one or two achromatic nucleoli, each enclosed in a large spherical vacuole. From this time onwards, until a

the final mitosis of the egg this V reappears, but it soon assumes the form of a straight rod which splits longitudinally, but in this case the halves remain united at one end, so that a *new* V is formed. The new V's now, in their turn, split longitudinally, the daughter-V's thus produced passing respectively to the egg and the second polar body.

much later stage, when the spermatid is approaching maturity, there is no very great alteration in the finer structure of the nucleus.

The two centrosomes have increased in size, and the peripheral one is seen to be flattened against the cell-membrane, forming the bottom of a slight funnel-shaped depression. A delicate thread, the axial filament of the tail, is now seen to project outward from the center of the disc-shaped centrosome. The two centrosomes are still connected, and the sphere, lying close to the nucleus, is joined to the deeper of the two by a narrow neck of sphere-substance.

Mid-bodies (*Zwischenkörper*) and sphere-bridges occur at this stage, and, so far as I am aware, this is the first case in which these structures have been observed in spermatids later than telophase. Only a small percentage of the spermatids show them, but, though not so well defined as those of the earlier generations, there can be no doubt of their nature. It is perhaps only in exceptional cases that they persist so late (small central-spindle bridges and mid-bodies are always visible in the dispireme stage), and I have constantly failed to discover them in more advanced stages. Some spermatids possess two bridges and mid-bodies, thus containing remnants of the last two divisions (see Pl. V, Fig. 21). In no case have I been able to trace actual connection of *spheres* of contiguous spermatids; and the fibers of the central-spindle bridge diverge toward the ends. The mid-body is split into two rings, one for each cell, precisely as in the earlier generations.

The structure which, following Meves, I have called the "sphere" is of astral origin (coming from astral-shield and remnants of old sphere), and is thus of the same nature as the sphere of a spermatogonium or spermatocyte. It is the same organ for which Meves (**'96a**) proposed the term "idiozome." The fact that the sphere in the spermatid does not surround the centrosomes may be accounted for by the circumstance that the centrosomes are attached to the cell-wall before the sphere is formed. The "achromatische Bestandtheil des Nebenkörpers" of Hermann's earlier paper on *Salamandra* (**'89**) is plainly the sphere; in his later work (**'97**) he calls it the

"ovaläre Körper" and describes it as surrounded by a granular "Archoplasma." He considers these structures to be of no farther use in formation of the spermatozoön.[1]

The sphere is probably of general occurrence in vertebrate spermatids, and in some cases it has been described as a "Nebenkern"; and it is also possible that some of the "Nebenkerne" and "Mitosomen" of the writers on spermatogenesis in invertebrates may in reality be spheres (*e.g.*, the smaller mitosome described by Platner, '89, in insects). A true "Nebenkern," derived from spindle fibers, seems not to occur in Amphibian spermatids.

In the stage last described, the more peripherally placed of the two centrosomes had assumed a disc-form, forming the bottom of a shallow depression in the cell-wall. A little later this disc-centrosome is seen to have given rise to a ring, which surrounds the axial filament, the filament itself now being attached to the deeper centrosome, which thus forms its "basal knob" or "end knob," a relation which henceforth persists permanently. I do not believe that any real transfer of attachment of the axial fiber occurs; before the ring is formed, the two centrosomes are united by a very short connecting piece; and if, as seems probable, the ring represents only the thickened and separated border of the disc, its center still exists to form a connection between the axial fiber and the deeper centrosome. After its separation the ring grows somewhat, and both it and the end-knob stain brilliantly, appearing black after iron-haematoxylin, and with the tar colors taking the plasma stains. The end-knob now becomes double, appearing as a minute dumb-bell-shaped body instead of assuming the form of a curved rod as in *Salamandra*.

During the differentiation of the ring, all the centrosomatic

[1] "Die Polstrahlung und die Mantelfaserung der karyokinetischen Spindel sind für die Thätigkeit des Spermatozoens bei dem Acte der Befruchtung werthlos geworden; sie sammeln sich deshalb, bereit zum Untergang, zu granulirten Massen im Leibe der Spermatide an. Wegen ihrer Beziehung zu der achromatischen Spindel habe ich sie oben mit dem Namen Archoplasma (Boveri) belegt; vielleicht dürfte es aber passender sein, auf sie den von verschiedenen Autoren schon gebrauchten Namen Mitosom (a term introduced by Platner) anzuwenden" (*l.c.*, p. 312).

organs — axial filament, end-knob, and ring — come to lie more deeply in the cytoplasm, partly, perhaps, because they really move nearer the nucleus, and partly from the fact that the cell-body elongates temporarily (Pl. V, Figs. 22 and 23). The slender cord of sphere-substance which extended to the deeper centrosome has disappeared, and the sphere is closely applied to the nucleus. Meves ('97) figures the ring as lying at the bottom of a long funnel, which is what would be expected, in view of its origin from the disc-centrosome. In *Amphiuma* a clear region, possibly representing such a tube, can be seen ensheathing the proximal end of the axial filament, but the cell-membrane lining the tube cannot be demonstrated after the ring is formed. Judging from Meves's figures, the funnel must be very distinct in *Salamandra*, but Hermann ('97) states that he has been unable to distinguish such a structure.

The above-described changes in the centrosomes correspond very closely in essential features with recent observations on *Salamandra* (Meves, '97), on mammals (von Bardeleben, '98, and Meves, '99), on elasmobranchs (Suzuki, '98), and even on the snail *Helix pomatia* (von Korff, '99). As long ago as 1889 Hermann figured the ring in *Salamandra* and suggested the origin of the axial filament from the other centrosome, though at that time he was not aware of the centrosomatic character of these bodies, and called them together the "chromatoide Nebenkörper." Flemming ('88) had previously asserted that the axial filament arose from the nuclear membrane, as has since been erroneously maintained by C. Niessing ('96) in rodents. The centrosomatic origin of the axial filament has now been definitely ascertained in Amphibia by Meves ('97) and Hermann ('97), in elasmobranchs by Moore ('95), Hermann ('97), and Suzuki ('98), in mammals by von Lenhossek ('97), Meves ('97), von Bardeleben ('98), and Benda ('98). Among invertebrates, the same has been found to be the case in gasteropods by Korff ('99), and in Hemiptera by Paulmier ('99).

That the spermatid ring is of centrosomatic origin cannot be questioned in the face of the abundant evidence above cited in amphibia, elasmobranchs, mammals, and gasteropods, but it is of interest to note the widely different origin which is attributed

to this structure in *Salamandra* and in Selachians in a recent article by so eminent an authority as Hermann ('97). This author holds that the ring is derived from the mid-body (*Zwischenkörper*) of the spindle of the last division (a view which Benda advanced some years previously, '93). The ring-shaped mid-body becomes double, and between its halves the spindle breaks in two. The half-spindle which each daughter-cell receives then assumes the form of a minute spindle, one pole being formed by the centrosome, the other by the half-mid-body. The spindle fibers now become compacted into a thread, which elongates to form the axial filament, thus explaining its fibrillar structure as described by Ballowitz ('90). The centrosome, of course, becomes the end-knob; and the mid-body, which has meanwhile resumed its peripheral location, again becomes ring-shaped, surrounding the axial filament.

These conclusions of Hermann, though appearing, *a priori*, most plausible, are clearly disproved by the conditions found in *Amphiuma*. Since *Amphiuma* shows the tail-filament projecting from the cell, while the sphere-bridges (remnants of the central spindle) are still visible, it is manifestly impossible for the axial filaments to represent coalesced spindle fibers ; and moreover two, and possibly more, bridges may enter a single cell. Hermann's derivation of the ring from a mid-body may be disposed of in the same manner, for (as shown in Pl. V, Fig. 21) the ring may be seen encircling the axial filament, while the same cell contains *two* ring-shaped mid-bodies, representing the "Zwischenkörper" of both maturation divisions.

In his criticism of Hermann's conclusions regarding the halving of the mid-body, Meves ('99) says : "Die Annahme aber, dass es sich theilt und die Theilstücke in die beiden Tochterzellen aufgenommen werden, ist rein hypothetisch." Though the halves do not enter the daughter-cells farther than to lie in the periphery, I have shown that in *Amphiuma* the mid-body does divide, somewhat as Hermann states, so that, in this point at least, his conclusions are not "rein hypothetisch." Hermann doubtless did see the split mid-body, which had the form of a ring ; his error lay in considering as the same structure the ring which later surrounded the axial filament.

A second account of ring formation in the Amphibian spermatid, which is widely at variance with the facts, is that of Bertacchini ('98), who ascribes to it a double origin — in part from two centrosomes and in part from the remains of aster and spindle.

B. *Later Changes.*

a. *Historical Discussion.* — After the differentiation of end-knob, axial filament, and ring, a series of phenomena ensues by which the apical body and the middle-piece of the spermatozoön are formed. Up to the present point my observations have agreed, in the main, with those of Meves ('96 and '97) on *Salamandra;* but from this point on, my conclusions, especially as regards the fate of the sphere and the origin of the middle-piece, are widely divergent from those of other observers, and in view of this fact it seems best to preface this portion of my account with a brief summary of the results obtained by others.

Flemming ('88) overlooked the sphere in the spermatid and considered the middle-piece as arising either from the chromatin of the nucleus, or as a local thickening of the nuclear membrane; while he regarded the apical body as a differentiation of the achromatic nuclear structure. Hermann ('89) describes the sphere as the "achromatische Bestandtheil des Nebenkörpers," but he held that it took no part in the metamorphosis, and early disappeared. His "chromatoider Nebenkörper" consisted of a ball and ring (the two centrosomes) lying in the cytoplasm. The "ball" became imbedded in the posterior end of the nucleus and enlarged to form the middle-piece. The axial filament grew out from the nuclear membrane at the point where the ball entered, and the ring elongated spirally around the axial filament to form a "Spiralfaden" (vibratile filament). The apical body he believed to be formed from the nuclear membrane.

In a later work ('97), Hermann still maintains that the "Archoplasma" (the achromatic Nebenkörper of his earlier paper, and really the sphere) is of no further use in the development of the spermatozoön. His view regarding the fate of the ring has been altered, and he now finds that the ring splits

into two, which encircle the axial filament. Of these he says: "Die Lagebeziehungen dieser beiden Ringe sind dabei solche, dass der proximale seine gewohnte Lage an der hinteren Peripherie des mächtig-herangewachsenen Mittelstückes innehält, während der distale sich caudalwärts gegen die Zellgrenze des Spermatidenleibes zu vorschiebt und dort einstellt. Zugleich lässt sich wahrnehmen, wie sich zwischen den beiden von einander weichenden Ringen eine zarte Substanzmenge ausgesponnen hat und als zarte Scheide den Anfangstheil des Schwanzfadens umgiebt. Dadurch, dass die beiden Ringe sich immer mehr von einander entfernen, wird natürlich diese Scheide stets grössere Strecken des Schwanzfadens umgeben müssen, zugleich aber wird auch der Protoplasmaleib der Spermatide bei den engen Beziehungen, in die die distale Ringbildung zu der Zellgrenze derselben getreten ist, immer mehr und mehr über den Schwanzfaden hinübergeschoben." Regarding the origin of the apical body, Hermann still holds to his original idea that it represents a differentiation of the nuclear membrane.

Bertacchini ('98), from studies of *Triton cristatus*, arrives at conclusions which are almost diametrically opposed to those of Hermann. He asserts that the ring is formed chiefly from remains of the spindle and asters, but that it contains two small centrosomes, which, undergoing certain peculiar evolutions, appear later at the base of the axial filament and finally become imbedded in the middle-piece. The middle-piece of the spermatozoön Bertacchini considers to be a metamorphosed mid-body (*Zwischenkörper*). In all these points he differs widely from other observers, and I feel sure from my own observations that little credence can be given to his conclusions.

The recent work of Meves ('97) on *Salamandra* is by far the most complete account yet published on the metamorphosis of the spermatid in Amphibia. His conclusions regarding the early differentiations of the centrosomatic organs have been cited above (p. 86), so that it is here only necessary to outline his description of the later transformations of centrosomes and sphere. Meves finds that the more deeply placed centrosome elongates to form a short curved rod, which approaches the

nucleus, and finally, becoming flattened against the nuclear membrane, sends a delicate plug (Zapfen) into the nucleus. This plug grows greatly, becoming first spherical, then cylindrical, and becomes the greater part of the middle-piece. (It will be noted that this is essentially in agreement with Hermann, though the details differ somewhat.) The ring centrosome elongates, becomes "pessary-shaped," and finally divides into two parts, one of which, the "dorsal" half, fuses with the anterior part of the middle-piece, becoming the "hintere Partie" of that organ, while the "ventral" half-ring moves along the axial filament almost to its extremity, forming a sort of rim to the advancing cytoplasmic mantle, which covers the ventral side (*i.e.*, the side opposite the vibratile membrane) of the axial filament. The elongating portion of the ring, between these two halves, fuses with the axial filament.

In regard to the origin of the apical body also, Meves's conclusions are at variance with those of the writers above cited, for he finds it to be derived from the sphere in the following manner. At a rather early stage, even before the peripheral centrosome has attained the ring form, the sphere becomes applied to the nuclear membrane. A number of minute vacuoles now appear in the sphere-substance, which later, by confluence, form one large vesicle, to the surface of which the remnant of sphere-material adheres. A little later this sphere-remnant disintegrates. The vesicle is seen to lie in a shallow indentation in the nuclear wall, and as the entire spermatid elongates, the vesicle breaks through the cell-membrane and comes to lie wholly outside the cell-body, though still attached to the nucleus. Later a small body appears between nucleus and vesicle, which Meves thinks probably arises from the latter. This body becomes conical, and its point gradually grows out through the sphere-vesicle, becoming the apical body or "Spiess." During the elaboration of the sphere-vesicle the entire sphere moves around the nucleus to a point opposite its original position, so that centrosomes and sphere are finally 180 degrees apart. Meves's account of the formation of apical body from sphere corresponds, in the main, to that first described by Benda ('91) in mammals, and since

confirmed, in the more important features, by Moore ('94) and Lenhossek ('98).

To sum up Meves's conclusions on these points: the sphere migrates through 180 degrees and gives rise to the apical body; a remnant left over soon disappears; the middle-piece is entirely centrosomatic, and is formed from the deeper centrosome and a portion of the ring centrosome; the remainder of the ring becomes extended along the axial filament.

b. *The Acrosome.* — In *Amphiuma* the stage shown in Fig. 22 shows a rudiment of the apical body. One or more small vacuoles appear within the sphere, and each, from its earliest appearance, is seen to contain a granule. Vacuoles and granules increase in size, and when there are several they soon become confluent and the central granules form one compact body. For this body in mammals Lenhossek ('98) proposed the term "Akrosom," and throughout the present paper I shall call it the *acrosome.* In some cases there is but one vacuole from the beginning, but frequently two or three are present, later forming one by coalescence. The vacuole at first lies at any point within the sphere, but later the single large vesicle comes to lie close against the nuclear membrane, and the enclosed acrosome lies at the nuclear side of the vacuole. Soon the vacuole becomes as large as the original sphere, apparently by imbibition of fluid, and the remnant of sphere-substance clings to its wall, usually as a lump on one side. It can now be seen that the vesicle rests in a shallow depression in the nuclear membrane, a condensation of chromatin forming a sort of collar around the area of union (Pl. V, Fig. 23). At this stage the two centrosomes (end-knob and ring) lie near the sphere, but are no longer connected with it. A zone of deeply staining granules surrounds the sphere, but it later disappears and I am unable to ascribe any significance to it. It resembles a sphere of microsomes which Niessing ('96) found surrounding the sphere in the rat-spermatid, and apparently represents the zone of granular "Archoplasma" which Hermann finds in Selachians and in the salamander surrounding the "ovaläre Körper" ('97).

It will be seen from this description that the vesicle and acrosome are metamorphosed from the sphere in substantially

the same manner as has been described by Benda, Moore, Meves, Lenhossek, and others. One point upon which observers disagree is the number of primary vacuoles — whether one or several; but *Amphiuma* shows that both modes may occur in the same species. Niessing ('96) believed that the "Mitosome" (acrosome) in the rat-spermatid was the centrosome, but this was disproved by Lenhossek ('98). Meves was unable to state definitely whether in the salamander the acrosome arose from nuclear membrane or sphere-vesicle, but he inclined, correctly, to the latter view. The acrosome is probably much smaller in *Salamandra* than in *Amphiuma*, and in *Cryptobranchus* and *Desmognathus* I have been unable to distinguish it until a much later stage, the early vesicle in these genera appearing empty, as figured by Meves.

When vesicle and acrosome have attained the condition shown in Fig. 23 a great change occurs in the relative positions of these structures and the centrosomes (*cf.* Figs. 23 and 25). The cytoplasmic layer covering the acrosome vesicle becomes thinner, and finally the vesicle projects through the cell-wall; or, in other words, the cell-membrane has become attached to the border of the area of contact of nucleus and vesicle. This is apparently produced by a shifting of the cytoplasm toward the opposite pole of the cell. The form of the nucleus changes also, becoming pyriform, with the smaller end toward the acrosome. Frequently, even before the nucleus loses its spherical form, a mass of dense chromatin is visible at the pole of the nucleus opposite the acrosome.

The most noteworthy change, however, at this time is the migration of the centrosomatic parts of the cell, together with the remnant of the sphere (the part left after acrosome vesicle is formed). These structures gradually move around the elongating nucleus until they occupy a position at the opposite end from the acrosome; *i.e.*, they migrate from the anterior to the posterior end of the nucleus (Pl. V, Figs. 24 and 25).

According to Meves, the centrosomes remain stationary and the vesicle migrates, but from a careful study of the question in *Amphiuma* I feel convinced that it is the acrosome vesicle which remains in its original place, while centrosomes migrate,

carrying with them the axial filament and the sphere-remnant. The acrosome vesicle has early broken through the cell-wall, and its point of attachment is marked by a pit in the nucleus and a collar of chromatin. Moreover, the opposite pole of the nucleus is early marked by the dense mass of chromatin above mentioned. Unlike the acrosome, the centrosomatic structures lie free in the cytoplasm, unless the ring is connected with the cell-wall, which cannot be demonstrated in *Amphiuma*. During the migration the centrosomes, and especially the axial filament, have the position relative to the pyriform nucleus which indicates that it is they, and not the acrosome, which migrate. Since the two poles of the nucleus are early marked by the acrosome and the dense chromatin-mass, it is plain that, if the fixed acrosome changes position, the entire nucleus must rotate, which is improbable. The fact that in the adult spermatozoa the acrosome is turned toward the wall of the cyst, while the spheres of the early spermatids were on the free side, is of no importance, for in the stages represented in Figs. 24 and 25 the spermatids are arranged with the utmost irregularity.

In brief, the attachment of acrosome to nucleus marks the anterior end of the spermatid from the start, and the lump of dense chromatin the posterior pole; a general flowing of cytoplasm toward the posterior end then occurs, which leaves the acrosome vesicle naked, while carrying with it the remnant of the sphere and the centrosomatic structures until these bodies come to lie at the posterior end of the cell, 180 degrees from the acrosome. This process is illustrated in Figs. 24 and 25. It may be mentioned here that Moore ('95) found that the sphere-vesicle in Selachians remained stationary, while the centrosomes migrated.

The later changes in the acrosome consist mainly in change of form, the vesicle finally becoming merely a sheath for the long conical acrosome, which projects anteriorly as a delicate thread. The apical barb, which is well developed in some urodeles (*e.g.*, *Salamandra* and *Desmognathus*), is very slight in *Amphiuma*.

c. *The Middle-piece.* — When this intra-cellular migration is accomplished, the spermatid enters a new phase — the forma-

tion of the middle-piece. The views of Hermann, Meves, and others have been stated above, and it is on this particular point that my observations are most at variance with theirs. Instead of its being formed entirely by metamorphosis of a centrosome, I find the middle-piece in *Amphiuma* to be chiefly derived from *the remnant of the sphere*, though the deeper centrosome (end-knob) does become imbedded in it.

As stated above, the remnant of the sphere, after the acrosome is formed, passes around the nucleus to its posterior end, together with the centrosomes, instead of disintegrating, as has been asserted. The sphere-remnant becomes closely appressed to the nuclear membrane at or near the point where is located the dense chromatin-mass marking the posterior pole. The centrosomatic structures also lie near this spot, the end-knob still maintaining its dumb-bell form. Under the mass of sphere-substance, — between it and the nuclear membrane, — a deeply staining mass now becomes visible, a mass which stains readily with the plasma tar colors (*e.g.*, orange or Congo red), but which also stains with iron-haematoxylin much more deeply than does the sphere. At first it is thin and flat, and may present the form of two or three minute flattened masses which fuse later (Fig. 26). The mass now increases in thickness, becoming hemispherical and finally globular. Since this structure appears between the sphere-substance and the nuclear membrane, it is impossible to state with absolute certainty from which it is derived, though the most probable explanation seems to be that it is *formed from the sphere-substance*, for the latter disappears as the globule enlarges. The globule continues for some time to lie outside the nuclear membrane, but a clear space, filled with karyolymph, soon appears under the membrane at the posterior end of the nucleus. This space is traversed by a thread which connects the globular body with the dense polar mass of chromatin (Pl. V, Figs. 28–30). During all these processes no change whatever is to be noted in the centrosomes; their position varies somewhat in different spermatids, but usually they lie close to the nuclear membrane, the dumb-bell-shaped end-knob almost or quite in contact with it. A slight, very diffuse rem-

nant of sphere-substance may be seen surrounding the globular body, but this soon disappears.

The next step is the passage of the globular body into the nucleus to form the middle-piece, and at the same time the basal knob (the deeper centrosome) becomes imbedded within the globular body (Pl. V, Fig. 31). These changes certainly take place very rapidly, though stages immediately before and immediately after the change are quite abundant. The spermatid shown in Pl. V, Fig. 32, exhibits the newly formed middle-piece, and the centrosomatic basal knob is visible as a highly refractile granule in its posterior part, immediately under the nuclear membrane. It is only in specimens stained so that the middle-piece has the proper degree of transparency that the centrosome granule can be demonstrated in the middle-piece.

The middle-piece appears to pass bodily through the nuclear membrane, and not to enter by any gradual flowing of its substance through the intra-nuclear thread (the "Zapfen" of Meves). At certain stages this thread appears more like a hyaline funnel or cylinder of fibers, and it is conceivable that the entrance of the middle-piece may be accomplished by the contraction of this structure. After its entrance the middle-piece grows very rapidly, becoming a short cylinder, and as the nucleus elongates and becomes thinner, nucleus and middle-piece are soon of equal diameter.

From a very early stage the dense polar mass of chromatin seems to be of some importance, and later it seems to determine the point of formation of the middle-piece, as if there were some form of "attraction" between it and the sphere-remnant and centrosomes. The ring centrosome still encircles the axial filament and lies close to the nuclear membrane.

The derivation of the middle-piece from a body other than the centrosome is widely at variance with the observations of most other investigators of vertebrate spermatogenesis, especially Hermann and Meves (see above, p. 90) and Suzuki ('98). Apparently the globular body is the body which Hermann and Meves consider a greatly increased centrosome, but since this body and the centrosome (end-knob) are visible at the same

time in *Amphiuma*, I am forced to the conclusions above stated. Notwithstanding the disparity between my conclusions and those of Meves, it seems that the former are more easily harmonized with the behavior of the middle-piece during fertilization (see above quotation from Fick, p. 64), and my observations regarding the division of the sphere to form both acrosome and middle-piece accord with those of Moore on elasmobranchs, and also with the behavior of the "Nebenkern" in mammals, as described by Benda. In invertebrates there is considerable evidence that the greater part of the middle-piece is not centrosomatic, though the centrosome lies within it.[1] In the earthworm Calkins ('95) has shown that the middle-piece is of archoplasmic (sphere) origin, and investigations by Field ('95) and Wilson ('99) have shown that in echinoderms the middle-piece during fertilization is left at the periphery of the egg as a useless remnant, so that in this case also it is non-centrosomatic.

The changes in the nucleus, by which it becomes a homogeneous rod of chromatin, the "head" of the spermatozoön, have been so fully described by others as to require no description here.

d. *The Tail of the Spermatozoön.* — Thus far the ring centrosome has remained inactive, lying close to the middle-piece, and still encircling the axial filament; it is surrounded by a thick layer of cytoplasm, but there are indications that the cell-membrane dips down, forming a funnel around the axial filament, so that probably the ring is in contact with the cell-wall at the bottom of a tube, as it was at the time of its formation. The ring now becomes obliquely placed and begins to elongate; one-half remains in place near the middle-piece, but the other advances toward the tip of the tail, exactly as described by Meves. At an early stage in the elongation the ring, viewed from the side, has the form of the letter *S* (Fig. 34). The elongating portion becomes very thin and finally becomes closely applied to the axial filament, though this does not occur so early as in *Salamandra*. During the elongation of the ring,

[1] v. Korff ('99) in *Helix pomatia* finds the entire middle-piece to be centrosomatic.

changes occur in the axial filament by which its cross-section becomes altered from a circle to the form illustrated in Pl. V, Figs. 37, *a–e*. Close to the middle-piece the filament becomes thicker, a groove appears along its "dorsal" surface, and farther along the groove becomes separated from the cylinder by a thin vertical plate. Toward the extremity the cylindrical part ends first, the grooved rod extending farther to form the "end-piece." At about the stage shown in Fig. 34 a vibratile membrane, bordered by a delicate vibratile filament, arises along the groove and extends the entire length of the tail. As the cytoplasmic mantle is not yet formed, this filament must be a differentiation of the axial filament. Meves found in *Salamandra* that the axial filament became a grooved rod, with a vibratile membrane growing from the groove, and for convenience he designated the grooved surface as "dorsal," but *Salamandra* and other urodeles which I have examined do not show the axial filament to be formed of a vertical plate bordered above by a groove and below by a cylindrical rod, as we find it in *Amphiuma*. The vibratile filament is much longer than the remainder of the tail, and is thrown into a series of waves.

As the ring elongates it can be seen that it is the "ventral" half which moves along the axial filament. It forms the border of the advancing cytoplasmic mantle, though the cytoplasm projects far ahead of it, forming a lobe which appears to surround the axial filament as a sheath. The advancing half-ring, seen through this projecting collar of cytoplasm, appears to be imbedded in cytoplasm, but such is not the case. The extended portion of the ring finally becomes fused with the lateral borders of the grooved dorsal part of the axial filament, so that the ventral portion of the filament is completely surrounded by the cytoplasmic mantle; but since the dorsal surface of the ring remains stationary, the grooved surface and the vibratile membrane remain exposed.

It has been possible to follow the progress of the mantle along the tail for a distance about equal to the length of the head of the spermatozoön, though it certainly extends farther. When it has attained that length, however, the ring is scarcely distinguishable, having become very thin and having to a great

extent lost its staining power. I believe that the entire ring eventually fuses with the axial filament after extending along a great part of its length. The dorsal half-ring does not split as it does in *Salamandra*, according to Meves, nor does it form a hinder segment of the middle-piece. When last distinguishable it is apparently fusing with the axial filament, close to the middle-piece. The cytoplasmic mantle becomes thinner as it elongates, and in mature spermatozoa a small lobe of granular cytoplasm may be seen attached to the ventral surface of the tail, but it becomes broken off bodily during the active movements of the cell. An excessively thin mantle which surrounds the head and middle-piece, and covers the ventral surface of the tail, is finally all that remains of the cytoplasm.

VI. Summary.

1. Amitosis occurs among primary spermatogonia and is, apparently, a normal process.

2. Secondary spermatogonia divide only by mitosis, and contain the somatic number of chromosomes.

3. During the growth-period certain changes occur in the chromatin, resulting in a synapsis, from which the chromatin emerges as a rough spireme consisting of only twelve threads (chromosomes), or one-half the "normal" number. In this spireme stage the cells (now primary spermatocytes) rest for a considerable period.

4. The twelve chromosomes split lengthwise, but the halves become reunited at the ends, forming ring-shaped chromosomes.

5. In the metaphase of the first maturation division (a heterotypical mitosis) the sister-chromosomes of a ring break apart at the original ends; and, at the same time, each V-shaped half splits longitudinally, so that each daughter-cell (secondary spermatocyte) receives twelve split V-shaped chromosomes.

6. In the very brief resting stage which ensues the chromosomes become indistinguishable.

7. In the prophase of the final (homœotypical) division twelve split V's appear, presumably those formed in the anaphase of the preceding mitosis. The sister-chromosomes of these split

V's separate, each daughter-cell (spermatid) thus receiving twelve single chromosomes.

8. There is thus no transverse or "reducing" division. Synapsis occurs before the first maturation division, and the chromatin is distributed by two longitudinal (equation) divisions.

9. The spheres of the resting secondary spermatocytes and spermatids differ from those of earlier generations in that the centrosomes, from the first, lie outside them.

10. Intercellular bridges (remnants of central spindles) and double ring-shaped mid-bodies occur in secondary spermatogonia, primary and secondary spermatocytes, and even in early spermatids.

11. The young spermatid has two peripherally located centrosomes and a sphere. The axial filament grows out from the centrosomes, one of which forms its end-knob, while the other forms a ring encircling it. The end-knob later assumes a dumb-bell form.

12. A portion of the sphere gives rise to the acrosome, and later the remaining portion, together with the centrosomatic structures, migrates to the opposite (posterior) pole.

13. The latter portion of the sphere becomes applied to the nuclear membrane, and gives rise to a body representing the rudiment of the middle-piece. The end-knob (centrosome) becomes imbedded in this body which passes through the nuclear membrane. The middle-piece thus arises from the sphere-substance, but contains a centrosome.

14. The axial filament gives rise to the vibratile membrane.

15. The ring centrosome becomes greatly extended along the axial filament, its "dorsal" half remaining in place while the ventral half marks the advancing border of attachment of the cytoplasmic mantle. The advancing half-ring never reaches the end of the tail-filament; its final position marks the limit of the mantle, and hence the juncture of the main part of the tail- and the end-piece. During the latter part of the elongating stage the ring stains very faintly. Ultimately it seems to fuse with the axial filament. A fusion of the dorsal half-ring with the middle-piece, such as Meves finds in *Salamandra*, does not occur in *Amphiuma*.

METHODS.

For fixation a number of methods were employed. In general the best results were obtained with material fixed in the well-known mixtures of Hermann and Flemming, though acetic-alcohol (70% alcohol two parts, glacial acetic acid one part) also proved to be valuable. The most satisfactory stain was Heidenhain's iron-haematoxylin, alone or followed by Congo red as a secondary stain. A dye which gives especially brilliant differentiation is the methyl-green and acid-fuchsine mixture of Auerbach. This stain does not always give satisfactory results with material fixed in osmic-acid mixtures, but successful preparations are very valuable, especially in the study of the cell-organs which participate in the formation of the middle-piece. The Biondi-Ehrlich and Flemming triple stains were also used, but gave, on the whole, less satisfactory preparations than were obtained by the stains before mentioned.

Nearly all the material was imbedded and cut in paraffine, though celloiden was also used to a slight extent, principally to discover, by comparison, whether the cell-structures suffered serious injury by the paraffine process. The comparison yielded nothing of importance, and I do not believe that amphibian testis material is injured by the paraffine process if fixation and dehydration have been thoroughly done.

For the examination of adult and nearly mature spermatozoa, material was obtained from the *vas deferens* and cut surfaces of the testis. This material was smeared thinly on the slide and fixed quickly with the various fixing fluids. Though quite successful with mature spermatozoa or advanced spermatids, this method has always proved a failure with spermatogonia and spermatocytes.

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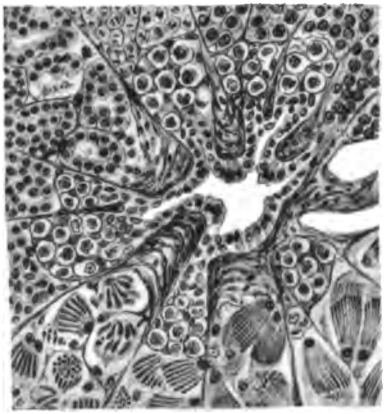

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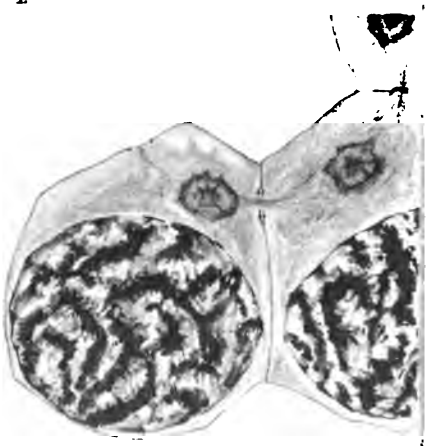

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EXPLANATION OF PLATES IV AND V.

[The figures, excepting Nos. 1, 2, and 37, were drawn with the aid of a camera lucida, using a Zeiss No. 6 compensating ocular and Zeiss apochromatic 2.0 mm. objective, apert. 1.30, with a tube length of 160 mm. All the figures are from *Amphiuma* except Nos. 6 and 8, which were drawn from preparations of *Spelerpes ruber*.]

FIG. 1. Central part of testis, in cross-section, showing germinal epithelium, and proliferation of spermatogonia. The central ends of "tubules," filled with various generations of cells, are shown.

FIG. 2. Primary spermatogonia, showing polymorphic nuclei, and amitotic division.

FIG. 3. Resting secondary spermatogonia, showing bridges and mid-bodies.

FIG. 4. Secondary spermatogonia in prophase. Formation of central spindle.

FIG. 5. Primary spermatocytes in resting stage. Chromatin in rough spireme, bridges and double-ring mid-bodies. Cells remain in this stage for a considerable period.

FIG. 6. Primary spermatocyte of *Spelerpes ruber*, showing effect of "osmication." Sphere appears vesicular.

FIG. 7. Primary spermatocyte. Spireme threads split. Centrosomes separated and connected by a centrodesmus.

FIG. 8. Same stage from *Spelerpes ruber*. Centrosomes separating inside the sphere.

FIG. 9. Primary spermatocyte. Formation of central spindle for first maturation division. Sphere still shows concentric layers.

FIG. 10. End of prophase. Seven of the twelve heterotype chromosomes are visible. The knobbed portions near the equator represent the ends of the sister-threads of a chromosome. The chromosomes at the extreme right and left show fusion of the sister-threads for some distance at both ends. The remnant of the sphere is still visible.

FIG. 11. Anaphase of heterotypical mitosis. In the chromosome at the extreme left the separation is not completed. Chromosomes all show the longitudinal split which gives them the split V-form.

FIG. 12. Secondary spermatocyte. Resting stage, showing rough spireme and sphere. This stage is of brief duration.

FIG. 13. Prophase of second maturation division (homœotypical). Chromosomes have the split V-form, as in the anaphase of the preceding division.

FIG. 14. Early anaphase of same.

FIG. 15. Late anaphase. Two cells of same stage, showing difference in form of spindle.

FIG. 16. Cross-section of anaphase (homœotypical mitosis), showing spindle-fibers in section. Ten of the twelve chromosomes are shown. Two of them show mantle-fibers attached.

FIG. 17. Telophase. Dispireme stage. Centrosomes have migrated and astral shields are formed. Central spindle persists as a bridge. Masses of sphere-substance (remnants of the old sphere) are visible.

FIG. 18. Stage intermediate between that of Fig. 17 and the spireme shown in Fig. 19.

FIG. 19. Spermatid with chromatin in rough spireme.

FIG. 20. Spermatid. Astral shield disappearing. New sphere now visible. Spireme is disintegrating.

FIG. 21. Three spermatids, showing intercellular central-spindle bridges and double-ring mid-bodies. The axial filament can be seen growing out from peripheral centrosome.

FIG. 22. The peripheral centrosome has become discoidal and lies at the bottom of a pit. A rudiment of the acrosome is visible in the sphere.

FIG. 23. The peripheral centrosome is now a ring surrounding the axial filament, while the deeper centrosome forms the end-knob. It is seen to have assumed a dumb-bell form. The large acrosome is attached to the nuclear membrane.

FIG. 23*a*. A section through the sphere of the same stage, including the acrosome. A zone of granules surrounds the sphere at this stage.

FIG. 24. The acrosome has come to lie outside the cell-body. The remnant of the sphere, together with the centrosomatic organs, is migrating to the opposite pole.

FIG. 25. This figure shows the migration about completed.

FIG. 26. The nucleus has elongated. The sphere-remnant is closely applied to the nuclear membrane, and a rudiment of the "globular body" is seen under the mass of sphere-substance.

FIGS. 27–30. Later stages, showing formation of "globular body." The appearance of a space under the nuclear membrane, and the thread connecting the "globular body" with chromatin, can be seen.

FIG. 31. The globular body passes through the nuclear membrane to become the middle-piece, while the end-knob (centrosome) becomes imbedded in it.

FIG. 32. Later spermatid, showing centrosome within the middle-piece. The acrosome has assumed the form of a long cone.

FIG. 32*a*. The "head" has become a homogenous rod of chromatin.

FIGS. 33 and 33*a*. The ring-centrosome elongating. "Dorsal" and profile views.

FIGS. 34 and 35. Later stages of the same in profile. The axial filament has altered in form, and the vibratile filament appears at this time.

FIG. 36. Late stage of elongation of ring. Dorsal view.

FIG. 37 *a*, *b*, *c*, *d*, and *e*. A series of cross-sections of the tail, "*a*" being nearest the middle-piece.

FIG. 38. Fully formed spermatozoön.

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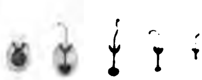

a b c d. e

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